The Crystal Structure of Porcine Reproductive and Respiratory Syndrome Virus Nonstructural Protein Nsp1{beta} Reveals a Novel Metal-Dependent Nuclease


Updated information and services can be found at: http://jvi.asm.org/cgi/content/full/84/13/6461

These include:

CONTENT ALERTS Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more>>
The Crystal Structure of Porcine Reproductive and Respiratory Syndrome Virus Nonstructural Protein Nsp1β Reveals a Novel Metal-Dependent Nuclease

Fei Xue,† Yuna Sun,† Liming Yan, Cong Zhao, Ji Chen, Mark Bartlam, Xuemei Li, Zhiyong Lou, and Zihe Rao

Structural Biology Laboratory, Tsinghua University, Beijing 100084, China; National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Science, Beijing 100101, China; and College of Life Sciences and Tianjin State Laboratory of Protein Science, Nankai University, Tianjin 300071, China

Received 8 February 2010/Accepted 12 April 2010

Porcine reproductive and respiratory syndrome virus (PRRSV), a member of the Arteriviridae family of Nidovirales, is the causative agent of porcine reproductive and respiratory syndrome, which results in enormous economic losses in the swine industry. As the second protein encoded by the PRRSV genome, nsp1β cleaves itself from the downstream nsp2 protein via a C-terminal papain-like cysteine protease (PCP) domain. Although nsp1β is known to be involved in virulence, its precise role in the process of viral infection remains unclear. In this work, we describe the homodimeric crystal structure of PRRSV nsp1β in its natural, self-processed form. We show that the architecture of its N-terminal domain (NTD) adopts a fold closely resembling that of several known nucleases and has intrinsic nuclease activity that is strongly activated by manganese ions in vitro. Key features, however, distinguish nsp1β from characterized nucleases, including the C-terminal PCP domain (which is responsible for the self-release of nsp1β from nsp2), a linker domain (LKD) that connects the NTD and the PCP domain, and a C-terminal extension (CTE) that binds to and is stabilized by the putative substrate binding site of the PCPβ domain. Combined with the reported nuclear localization of this protein, these results shed light on the self-processing mode and precise biological function of nsp1β and thus offer a multitarget template for future drug discovery.

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiological agent responsible for a disease in pigs called porcine reproductive and respiratory syndrome (PRRS), which causes severe reproductive failure in sows. PRRS is characterized by late-term abortion, early farrowing, stillbirth, and the birth of weak piglets; it is also associated with the porcine respiratory disease complex in combination with secondary infections (43). Initially referred to as “mystery swine disease,” “mythic reproductive syndrome,” and “blue-ear disease,” it was first reported in 1987, from North America (1) and central Europe (29). From 2006 to 2008, a PRRS epidemic occurred in China and Southeast Asia, where it was initially known as “high fever disease.” Thus, PRRSV has been found in the majority of swine-producing countries around the world and has caused enormous economic losses in the swine industry (24).

There are two prototype PRRSV strains: the North American strain is VR-2332, and the European strain is the Lelystad virus (LV). Although these two strains cause similar clinical symptoms, they represent two distinct viral genotypes, with their genomes diverging by around 40% (37). Unfortunately, the genetic variation among viruses isolated from different locations (17, 25) increases the difficulty of developing effective vaccines.

PRRSV is a small, enveloped RNA virus belonging to the genus Arterivirus in the family Arteriviridae of the order Nidovirales (33). The three other known members of this genus are Equine arteritis virus (EAV), Simian hemorrhagic fever virus (SHFV), and Lactate dehydrogenase-elevating virus (LDV). PRRSV contains a single-stranded, positive-sense RNA genome (~15 kb) that carries nine open reading frames (ORFs). Following virus entry into cells and release of the genome into the cytoplasm, the PRRSV life cycle begins with expression of the large replicase gene, consisting of ORFs 1a and 1b. Genome translation yields two multidomain replicase polyproteins, named pp1a and pp1ab, with the latter being a C-terminally extended version of the former due to a ribosomal frameshift mechanism (19). Polyproteins pp1a and pp1ab are predicted to be cleaved into 14 nonstructural proteins (nsps) by the nsp4 3C-like main protease (38) and three accessory proteinases residing in nsp1 (including nsp1α and nsp1β) and nsp2 (41, 50) (Fig. 1). The resulting mature nsps direct viral RNA synthesis, presumably after forming a replication-transcription complex (RTC) that is associated with endoplasmic reticulum-derived paired membranes and double-membrane vesicles (10, 30).

Nsp1 is reported to be a multifunctional protein containing two papain-like cysteine proteases, named PCPα and -β, and a zinc finger motif required for subgenomic mRNA (sg mRNA) transcription. From sequence analyses, it was determined that nsp1α contains the N-terminal zinc finger and PCPα, while nsp1β contains PCPβ (6, 27, 39, 40). Although PRRSV nsp1α...
and -β share low sequence identity with papain, the characteristic residues of papain-like proteases are generally conserved in the primary sequences of the nsp1α and -β proteins from EAV, PRRSV, LDV, and SHFV. Nsp1α and -β are the first two self-releasing proteins encoded in the PRRSV polyprotein. They cleave themselves from downstream proteins through their C-terminal PCP domains, at Cys-Ala-Met180 (the residues are numbered according to the nsp1α and -β amino acid sequences, respectively) cleavage sites (34).

Previous studies have shown that mutations that inactivate PCP activity completely block sg mRNA synthesis, while PCPβ activity was shown to be essential for viral RNA synthesis. This indicates that the correct processing of the nsp1α-nsp1β and nsp1β-nsp2 cleavage sites is essential for PRRSV genome replication (19). Moreover, it was previously suggested that the PCPβ domain interacts with p100, a cellular transcription cofactor, and thus affects sg mRNA synthesis much like its homolog in EAV (39). Furthermore, nsp1β was reported to be a determining factor in the virulence of PRRSV (20). While this report was in preparation, Beura and colleagues reported that nsp1β-mediated subversion of the host immune response plays an important role in PRRSV pathogenesis (2). Despite the crucial role that nsp1β plays in the PRRSV life cycle and its virulence, the self-processing mode and precise functional role of nsp1β remain unclear due to a lack of direct evidence. Additionally, the differences between the self-processing modes of several reported viral PCP proteins, including foot-and-mouth disease virus (FMDV) Lpro, PRRSV nsp1α, and PRRSV nsp2, veil the precise self-processing mode of PRRSV nsp1β (11, 12).

We report here the three-dimensional structure of PRRSV nsp1β. The C-terminal domain of nsp1β adopts a conformation similar to that of other proteases in the papain superfamily. This is consistent with reported results, and moreover, the structure shows the exact cleavage site, at Trp-Tyr-Gly203 in Ala-Gly-Lys. Surprisingly, the crystal structure revealed that the N-terminal domain (NTD) of nsp1β has a strict metal-dependent nuclease activity (demonstrated by in vitro nucleic acid degradation assays). Furthermore, Lys18 and Glu52, which are located in the charged area on the surface of the nsp1β NTD, were confirmed to be crucial for nsp1β nuclease activity by mutagenesis study. Combined with the recently reported result showing nuclear localization of nsp1β in infected cells (2, 4), these data allow us to understand the precise functional role of nsp1β in PRRSV infection and thus offer a multitarget template for future drug discovery.
performed with CNS (3). During the later stages of positional refinement, restraints were relaxed, and a bulk solvent correction was applied under the guidance of $R_{	ext{free}}$. Model geometry was verified using the program PROCHECK (21). Solvent molecules were located from stereochemically reasonable peaks in the $\sigma$-weighted $F_o-F_c$ difference electron density map. Final refinement statistics are shown in Table 1. Figures were created using PYMOL (5).

### TABLE 1. Data collection and refinement statistics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peak</th>
<th>Edge</th>
<th>Remote</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collection statistics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell parameters</td>
<td>$a = b = 51.0$, $c = 154.6$</td>
<td>$a = b = 51.0$, $c = 154.6$</td>
<td>$a = b = 51.0$, $c = 154.6$</td>
</tr>
<tr>
<td>Space group</td>
<td>$P4_12_12$</td>
<td>$P4_12_12$</td>
<td>$P4_12_12$</td>
</tr>
<tr>
<td>Wavelength used (Å)</td>
<td>0.9798</td>
<td>0.9795</td>
<td>0.9600</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>50.0 (2.9)–2.8</td>
<td>50.0 (2.9)–2.8</td>
<td>50.0 (3.1)–3.0</td>
</tr>
<tr>
<td>No. of all reflections</td>
<td>61,067</td>
<td>60,801</td>
<td>126,510</td>
</tr>
<tr>
<td>No. of unique reflections</td>
<td>8,281</td>
<td>8,263</td>
<td>10,428</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>95.3 (95.1)</td>
<td>99.5 (99.9)</td>
<td>93.7 (8.5)</td>
</tr>
<tr>
<td>Average $I/\sigma(I)$</td>
<td>35.3 (2.3)</td>
<td>35.7 (2.2)</td>
<td>6.0 (3.2)</td>
</tr>
<tr>
<td>$R_{	ext{merge}} %$</td>
<td>6.5 (41.9)</td>
<td>6.6 (43.6)</td>
<td>11.3 (56.3)</td>
</tr>
</tbody>
</table>

Refinement statistics

- No. of reflections used [$\sigma(F) > 0$] | 8,269 |
- $R_{	ext{work}} \%$ | 22.5 |
- $R_{	ext{free}} \%$ | 27.1 |
- RMSD bond distance (Å) | 0.006 |
- RMSD bond angle (°) | 1.452 |
- Average B value (Å$^2$) | 58.3 |

Ramachandran plot (excluding Pro and Gly)

- No. (%) of residues in most-favored regions | 115 (81.0) |
- No. (%) of residues in additionally allowed regions | 22 (15.5) |
- No. (%) of residues in generously allowed regions | 5 (3.5) |

#### RESULTS AND DISCUSSION

### Structure of PRRSV nsp1β

The crystal structure of the 23-kDa PRRSV nsp1β protein (residues Ala1 to Gly203) was determined using the MAD method and refined to 2.8-Å resolution with a final $R_{	ext{work}}$ value of 22.5% ($R_{	ext{free}} = 27.1\%$). The crystal belongs to the space group $P4_12_12$ and there is one nsp1β molecule in an asymmetric unit with a Matthews coefficient of 2.2 Å$^3$/Da (corresponding to 46% solvent content) (23). The nsp1β monomer possesses an overall elliptical structure with dimensions of 50 by 35 by 30 Å and consists of six α-helices and seven β-strands (Fig. 2A). It can be divided into two major parts: the N-terminal domain (NTD; Ala1 to Ser48) and the known C-terminal papain-like cysteine protease domain (PCPβ; Val85 to Pro181). The structure also features two accessory regions: the linker between the N- and C-terminal domains (LKD; Phe49 to Thr84) and a C-terminal extension
and α3, and from the loop region which connects β5 and β6 in the PCP domain. These residues include Asp2, Val3, Tyr4, Pro23, Arg24, Glu28, Lys30, Phe31, Glu32, His52, Lys153, Glu154, Trp156, and Lys200, together with their counterparts in the second monomer (Table 2). The relatively hydrophilic contact region between the two monomers is 3,145 Å² (31% of the total) from each monomer, consistent with the strong dimerization in solution (Fig. 2F and G). In addition, attempts to construct a series of point mutations that abolish dimerization failed when the expressed proteins were found to be insoluble. This was putatively due to unfavorable solvent-exposed surfaces, which is very similar to what was found for the crystal structure of PRRSV nsp1α (34). Together, both the structural and solution data suggest that a homodimer is likely to be the biologically functional unit of nsp1β.

While the NTD and certain regions of the PCP domain of nsp1β interact with each other to form the homodimer, the PCP enzyme active sites of each subunit within the homodimer are on opposing sides and are exposed to solvent. This permits independent function of the active sites (if they are not blocked by the CTE) and suggests a cis self-processing mode. It should be noted that according to a comparison of the crystal structures of PRRSV nsp1α and FMDV L\(^{195}\), FMDV L\(^{190}\) functions as a monomer when it proteolytically cleaves the host cell protein eIF4G (11), while PRRSV nsp1α likely also forms a dimeric biological unit (unpublished data). Although the PCPβ domain of PRRSV nsp1β shares high structural similarity with other viral papain-like proteases, its NTD is quite different. These additional structural features, including the NTD and the dimeric architecture of PRRSV nsp1β, may bear important functions in addition to the known protease activity.

**The structure of the nsp1β NTD reveals a nuclease activity.** Although comparisons with the entire nsp1β monomer yielded no distinct structural homologues, comparisons of the NTD

---

**FIG. 2.** Crystal structure of PRRSV nsp1β. (A and B) Overall structure of the nsp1β monomer and topology. The monomer structure of nsp1β is shown as a colored ribbon. The NTD, LKD, PCP domain, and CTE are colored green, red, blue, and gold, respectively. The topology of nsp1β is colored using the same scheme. The α-helices and β-strands are shown as bars and arrows, respectively. (C) Gel filtration of nsp1β. An nsp1β solution (0.5 ml at 2 mg/ml) was loaded onto a Superdex 200 column (GE Healthcare) and eluted with a buffer containing 50 mM MES (pH 6.0) and 1 M NaCl. (D) Cross-linking gel with nsp1β. See Materials and Methods for experimental details. Each lane and the standard protein markers are labeled. (E) Side and top views of the nsp1β homodimer. The structure is shown as colored ribbons. The two monomers in one homodimer are colored blue and cyan. The 2-fold axis, which relates the two monomers, is labeled. (F) The residues which contribute to intermonomer interactions are labeled. The nsp1β molecule is shown as a blue ribbon covered with a transparent white surface. The major residues which contribute to intermonomer interactions (distances of <3 Å) are colored red, while the other residues are colored gold. (G) The surface potential of the nsp1β interaction.
and PCPB domains with other structures in the Protein Data Bank (PDB) (using the structure comparison service SSM [18]) yielded several structural homologues, from the endonuclease and cysteine protease families, respectively. PCPB has been demonstrated to possess papain-like cysteine protease activity related to the self-release of nsp1β, and thus the SSM result for the PCPB domain was totally unsurprising. Interestingly, however, the most significant matches to the nsp1β NTD, based on the highest Z and Q scores, included the reported structures of the lambda integrase N-terminal domain (PDB accession code 1KKJ [45]) (Z score, 4.7), the TN916 integrase N-terminal domain (PDB accession code 1TN9 [44]) (Z score, 4.6), the methyl-CpG-binding domain of human MBD1 (PDB accession code 1IG4 [26]) (Z score, 5.3), and the endoribonuclease Sa (PDB accession code 1ZGX [31]) (Q score, 0.15). Moreover, the topology of HIV-1 integrase was also found to share similarity with the NTD of PRRSV nsp1β. Although the sequence identities of nsp1β with HIV-1 integrase and RNase Sa are relatively low (10% and 11%, respectively), secondary structural superposition revealed that the structures of their core regions are remarkably similar, with root mean square deviations (RMSD) of 2.8 Å and 2.0 Å, respectively (Fig. 3A). Since HIV-1 integrase is also known to have nonspecific nuclease activity (9), all of these structural features suggest a putative nuclease activity for the nsp1β NTD.

Since many nuclease are reported to be dependent upon divalent cations for their activity (7, 14, 22, 35, 48, 49), we performed an in vitro nucleic acid degradation assay to further examine this potential nuclease activity (and possible divalent metal ion dependence) of nsp1β. The results show that nsp1β does have intrinsic nuclease activity that is strictly divalent cation dependent (Fig. 3B, C, and D). The PRRSV nsp1β nuclease activity (using λ-DNA as a substrate) was enhanced in the presence of either Mn2+ or Mg2+ ions but not Ca2+ or Ni2+ ions (Fig. 3C). Furthermore, the nuclease activity was greatest in the presence of Mn2+ ions rather than Mg2+ ions, and the substrate degradation pattern with equimolar amounts of Mn2+ and Mg2+ ions is distinguishable from that with Mn2+ ions alone (Fig. 3C). To examine the nuclease activity of PRRSV nsp1β on other types of nucleic acid, we also performed the in vitro nucleic acid degradation assay by using rRNA extracted from fresh porcine liver (ssRNA) and synthesized ssDNA and dsRNA as the substrates. Though PRRSV nsp1β showed distinct nuclease activity on ssRNA, the same as that with dsDNA, it unexpectedly and completely lost this nuclease activity with ssDNA and dsRNA as the substrates (Fig. 3E and F). While this report was in preparation, the reported results of subcellular localization experiments in MARC-145 cells showed the nuclear localization of nsp1β molecules in infected cells (2). Taken together, these results suggest that the NTD of nsp1β presents a nuclease activity on ssRNA and dsDNA, and we propose that this ability could be related to its virulence determinant role in PRRSV infection by affecting the nucleic acids in host cells.

In the crystal structure, the six NTD β-strands in the interacting region of the monomer combine to form a barrel-like structure. The Ala1-Val3, Tyr13-Trp27, and His52-Val55 residues of one monomer face their counterparts in the neighboring monomer to form an S-shaped, positively charged pocket with a diameter of ~10 Å and a depth of ~12 Å extending to ~30 Å (Fig. 4A). The properties of this pocket suggest a putative nucleotide binding site which could be related to nuclease activity. In particular, Lys18 is solvent exposed and might help to stabilize bound nucleic acid. Moreover, since acidic residues are usually found in the active sites of nucleases, the negatively charged region formed by Asp2, Glu32, and Glu154 in the potential surface of the nsp1β NTD could also be related to nuclease activity. Additionally, all of the residues that are involved in the putative nucleic acid binding groove are conserved among the different PRRSV strains, suggesting similar nuclease activities (13). To find the key residues which are crucial for nsp1β nuclease activity, a series of mutants was verified by nucleic acid degradation assay. Unsurprisingly, mutation of Lys18 and Glu32, located in the positively and negatively charged regions, respectively, specifically abolished the nuclease activity of nsp1β, supporting our hypothesis (Fig. 4B).

Unfortunately, attempts to solve the crystal structure of PRRSV nsp1β in complex with substrates, metal ions, or potential inhibitors failed and prevented us from describing the clear scenario of nsp1β nuclease activity. Nevertheless, the results from the structural investigation and in vitro nucleic acid degradation assays provide a solid basis to begin to understand the functional role of PRRSV nsp1β and to formulate an anti-PRRSV therapy. It is conceivable that nucleotide analogues which bind to the nsp1β nuclease active site with high affinity, and thus act as competitive inhibitors of the enzyme, can be employed as therapeutics to combat the current threat of highly pathogenic PRRSV.

The active site of PCPB and its interaction with the CTE. The structure of the PCPB domain in the C terminus of nsp1β helps to illustrate the autocleavage site for nsp1β/nsp2 processing. PRRSV nsp1β frees itself from the parent polypeptide chain by cleavage between its own C terminus and the N terminus of nsp2, the downstream protein in the polypeptide. To further confirm the exact cleavage site of nsp1β, we constructed and expressed an nsp1β-nsp2K122 fusion in order to determine the N-terminal sequence, which is similar to that described for nsp1α (unpublished data). The results show that the exact self-cleavage site of nsp1β is Trp-Try-Gly203 | Ala-Gly-Lys, which is consistent with previous reports (19). In the final refined nsp1β model, the last residue (Gly203) has high-quality electron density in the vicinity of the PCPB catalytic center.

In the catalytic center, one of the carboxylate oxygen atoms of Gly203 (usually named the P1 substrate residue) points toward the “oxyanion hole” and is stabilized by the sulphydryl group of Cys90 and the imidazole group of His159, with distances of 2.1 Å and 2.5 Å, respectively. This is very similar to the substrate binding behavior in the crystal structure of PRRSV nsp1α. As with previously reported crystal structures of PCPs, His159 initiates a hydrogen bonding network involving Gln148 and Leu160 that might act as a charge relay system during catalysis (Fig. 5B).

The presence of the CTE residues running along the substrate binding groove and their stabilization by a network of hydrogen bonds suggest the possibility of the same substrate binding during self-processing. The last six residues of the CTE are in an extended conformation (Fig. 5A, B, and C), similar to that observed in complexes of enzymes of the papain super-
family with peptide-like inhibitors (46, 47). Three major parts of the PCPβ domain contribute to CTE binding. Residues on helix α4 (Lys124 to Arg128) form one wall of the binding pocket, while residues on strands β5 and β7 form the other wall (Fig. 5A). The central helix (α2) and several side chains on β6 form the bottom of the binding pocket. The two walls sandwich the CTE tightly to stabilize its conformation, and the catalytic center is located at the top of the substrate binding pocket.

On the CTE side, the major interactions between the CTE and PCPβ are essentially hydrophobic and provided by Phe194 to Gly203. Whereas Gly203 occupies the S1 position, Tyr202, Phe196, and Phe194 occupy the S2 to S4 subsites, respectively. Unlike some reported structures of PCPs and their substrate
A possible self-processing mode. Although there is still a lack of sufficient biochemical data to firmly establish a model for the mechanism of protease self-processing, several structural features of PRRSV nsp1β suggest that a cis (intramolecular) processing mode may be favored for the self-release of nsp1β from nsp2. In our crystal structure, the residues N-terminal to the cleavage site were well defined in the electron density map and were well stabilized by an intramolecular hydrogen bond network. Notably, as discussed above, one carboxylate oxygen atom of the C-terminal Gly203 residue points toward the oxyanion hole and is stabilized by the sulfhydryl group of Cys90 and the N61 atom of His159. This oxygen atom likely represents the position of the actual product after hydrolysis, and Gly203 fully occupies the nsp1β active site. Moreover, an in vitro enzyme assay using a fluorescently labeled substrate indicated no distinct nsp1β protease activity, which was unsurprising based on the very stable binding behavior of Gly203. In addition, the cleavage sites of the two subunits are on opposite sides of the homodimer. Considering the peptide length of the CTE region, it is unlikely that the two subunits could self-process in trans. Therefore, intramolecular self-processing seems the most probable mechanism for PRRSV nsp1β, based on our current model. The same observation was also made with the crystal structure of PRRSV nsp1α, and similar conclusions have thus been drawn for PRRSV nsp1α and FMDV Lpro, for which cleavage in cis is considered the most probable scenario. The binding in trans of the CTE domain observed in the FMDV Lpro crystal structure is an artifact of crystallography and its host cell eIF4G cleavage ability (11).

From sequence alignments of PCPβ domains, we found that the nsp1β proteins of PRRSV, LDV, SHFV, and FMDV Lpro share high similarity (Fig. 5D). For example, aside from the catalytic dyad (Cys90 and His159, which correspond to Cys276 and His345 in a previous report [16]), Try91, covering the hydrogen bond between the dyad, is also conserved, indicating a similar cleavage mechanism shared by the PCPβs of PRRSV, LDV, and SHFV.

By comparing our data with the PCP domains in FMDV Lpro and severe acute respiratory syndrome coronavirus (SARS-CoV) PLpro, we found that there are distinct differences between the PCP domains in these three crystal structures. In the FMDV Lpro structure, one of the two CTEs in each asymmetric unit is in a free and flexible state, while the other is well stabilized by the PCP domain of an adjacent Lpro molecule. This observation illustrates that the contact between the FMDV Lpro CTE and the PCP catalytic domain is not very stable in solution, consistent with its alternative proteolytic ability toward eIF4G. A similar observation was also seen in the crystal structure of SARS-CoV PLpro. No substrate was found in the active site of its PCP, which is consistent with the deubiquitinating enzyme activity of PLpro. In sharp contrast, the CTE of PRRSV nsp1β is well stabilized by the PCP domain and is unlikely to allow subsequent protease activity on other substrates after self-cleavage. This product-inhibitor phenomenon suggests that the PCP domain of PRRSV nsp1β is unlikely to have specific proteolytic activity toward any host cell protein. It further suggests that self-release of nsp1β from the polyprotein is directly related to some nonproteolytic activity (40).

Conclusions. PRRSV nsp1β is the second protein in the pp1a polyprotein encoded by PRRSV and has been reported to be essential for viral RNA synthesis and virulence. The crystal structure of nsp1β consists of the NTD, which contains a strict metal ion-dependent nuclease activity on ssRNA and dsDNA; a C-terminal PCP domain, which can release the complexes, the CTE (which behaves as the substrate of PCPβ) does not extend and bind linearly in the substrate binding groove. Gly197 to Trp201 are surrounded by the hydrophobic side chains of Leu94, Leu126, Leu130, Leu135, Ile147, Leu160, and Ile177. Thus, the corresponding S1 and S2 sites of the PCPβ domain are mostly hydrophobic subsites with a vacant volume of ~450 Å³. The hydrophobic side chain of Phe196 from the CTE is completely buried in the S2 subsite. This hydrophobic subsite is relatively similar to, although not strictly conserved with, the equivalent subsite in most papain-like proteases. The S2 subsite lies at the end of the binding pocket and is occupied by Phe194. In addition, residues of the CTE also establish a number of hydrogen bonds with the PCPβ domain to stabilize its conformation (Table 3).
FIG. 5. PCPβ active site and interaction with the CTE. (A and C) Interaction between the CTE and PCPβ. The CTE is shown as colored sticks, while the other parts of nsp1β are shown as the molecular surface. The left and right walls are colored red and blue, respectively. The residues that contribute to the CTE-PCPβ interaction are labeled. (B) Hydrogen bond network around His159. Residues are colored according to their domain association (blue for PCPβ and yellow for CTE). Hydrogen bonds are shown as red dashed lines and labeled with the distance. (D) Sequence alignment of nsp1β proteins in PRRSV, LDV, SHFV, and EAV. Key residues for the proteolytic activity of the papain-like cysteine proteases and cleavage sites are framed with red and blue rectangles, respectively. Secondary structure elements of PRRSV nsp1β are marked at the top of the alignment; α-helices and β-strands are presented as loops and arrows, respectively.
protein from the downstream nsp2 protein; a CTE, which exemplifies the substrate binding to the proteolytic catalytic site during self-processing; and a linker connecting the NTD exemplifies the substrate binding to the proteolytic catalytic of nsp1 modimer formation. Moreover, the homodimeric architecture of different PRRSV prototype strains. Nuclelease activity during viral replication and infection expands dual role of PRRSV nsp1/H9252

2008AA000238), and Tsinghua University Initiative Scientific Re-
National Major Projects (grants 2009ZX09311-001 and 2009ZX10004-973 and 863 Projects (grants 2006CB806503 and 2006AA02A322), This work was supported by the Ministry of Science and Technology We thank M. Liao for providing PRRSV XH-GD genomic DNA
Porcine reproductive and respiratory syndrome virus nonstructural protein 1

Pattnaik, and F. A. Osorio.
8th ed. Iowa State University Press, Ames, IA.
34. Sun, Y., F. Xue, Y. Guo, M. Ma, N. Hao, X. C. Zhang, Z. Lou, X. Li, and Z. 
Rao. 2009. Crystal structure of porcine reproductive and respiratory syn-
Williams, and J. A. Grasby. 2008. Three metal ions participate in the reac-
37. Thiel, H. J., G. Meyers, R. Stark, N. Tautz, T. Rumenapf, G. Unger, and 
viruses: pestiviruses and the porcine reproductive and respiratory syndrome 
Yan, R. Hilgenfeld, and G. F. Gao. 2009. Structure and cleavage specificity of 
the chymotrypsin-like serine protease (3CLSP/nsp4) of porcine reproductive 
protein 1, an essential factor for viral subgenomic mRNA synthesis, interacts 
with the cellular transcription co-factor p100. J. Gen. Virol. 84:2317–2322.
zinc finger-containing papain-like protease couples subgenomic mRNA syn-
2006. Proteolytic maturation of replicase polyprotein pp1a by the nsp4 main 
proteinase is essential for equine arteritis virus replication and includes 
42. Van Duyne, G. D., R. F. Standartaert, P. A. Karplus, S. L. Schreiber, and J. 
Claridy. 1993. Atomic structures of the human immunophilin FKBP-12 com-
2009. Development of an experimental inactivated PRRSV vaccine that 
44. Wojcik, J. M., K. M. Connolly, and R. T. Clubb. 1999. NMR structure of the 
papain-succinyl-Gln-Val-Ala-Ala-p-nitroanilide complex at 1.7-A resolu-
tion: noncovalent binding mode of a common sequence of endogenous 
46. Yamamoto, D., K. Matsumoto, H. Ohishi, T. Ishida, M. Inoue, K. Kitamura, 
47. Yang, W. 2008. An equivalent metal ion in one- and two-metal-ion catalysis. 
48. Yuan, P., M. Bartlam, Z. Lou, S. Chen, J. Zhou, X. He, Z. Lv, R. Ge, X. Li, 
influenza polymerase PA(N) reveals an endonuclease active site. Nature 
458:909–913.
49. Ziebuhr, J., E. J. Snijder, and A. E. Gorbalenya. 2000. Virus-encoded pro-
879.